Antimicrobial and antioxidant activities of three *Euphorbia* species

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Abstract Three toxic plants belonging to *Euphorbia* genus, namely *Euphorbia biumbellata*, *Euphorbia terracina* and *Euphorbia dendroides* were phytochemically screened and their total phenolic compounds (TPC) and total flavonoid compounds (TFC) were measured and antioxidant capacities were evaluated by DPPH test expressed by Vitamin C Equivalent Antioxidant Capacity (VCEAC). The VCEAC values for the three medicinal plants ranged from 1.0655 g to 0.7285 g of vitamin C equivalents (VCEAC)/100 g of dry matter. The concentrations of TPC and TFC in three plants were respectively 15.13 g to 7.55g of gallic acid equivalents (GAE)/100 g of dry matter and 7.06 g to 5.8 g of quercetin equivalents (QE)/100 g of dry matter. The methanolic extracts of these plants were used to determine their antibacterial activity with the disc diffusion method. The results showed that the three plants were active with the inhibition zones obtained varying from 8 to 22 mm. The highest inhibition zones reached was 22 mm for *Euphorbia biumbellata* leaves against *Staphylococcus aureus*. Phytochemical analysis of the total extract showed the presence of biologically active compound groups such as polyphenols, flavonoids, glycosides and tannins.

Keywords *Euphorbia*; antioxidant; polyphenols; flavonoids; DPPH; antimicrobial.

INTRODUCTION

Poisonous plants contain bioactive compounds with biochemical effects in man and animals. These compounds have various and conflicting pharmacological properties, from being acute deadly to being curative. Then, this impact on the health has been reported and associated to antioxidant compounds [1].

In this work, we targeted three plants growing in north-east of Algeria. The selected plants are *Euphorbia biumbellata*, *Euphorbia terracina* and *Euphorbia dendroides* which are poisonous (The whole aerial parts: leaves, stem and fruits) [2], but known for their uses in traditional medicine for various diseases.

Euphorbia species have been reported to have medicinal activity as anticancer and against certain viruses, bacteria and fungi [3-8]. Latex of some species used in folk medicine as topical treatment in some skin diseases and sexual transmitted disease like gonorrhea and in case of migraines and gastric parasite [9].

According to ethnobotanical investigations, it is observed that in some regions of Algeria, *Euphorbia terracina* and *Euphorbia biumbellata* have been used for treating warts by latex found in their stems [9-12]. Few previous works are reported on chemical composition of *Euphorbia terracina* aerial parts, but there are still no phytochemical and biological studies about of aerial parts of *Euphorbia biumbellata* and *Euphorbia dendroides.*

To our knowledge, there are no previous reports concerning *in vitro* antioxidant activities of these plant part extracts. The purposes of this study were to evaluate the antimicrobial capacity and to determine the total phenolic and the total flavonoid contents, to evaluate their antioxidant activities using 2,2- diphenyl-1-picrylhydrazyl (DPPH) test.

MATERIAL AND METHODS

Plant materials

E. biumbellata, E. dendroides and *E. terracina* were collected from National Park of El Kala (extreme North-east of Algeria) and during the flowering stage in March 2012 and they were identified by Dr. Georges De belair (Department of Biology, University of Badji Mokhtar-Annaba, Algeria). Voucher specimens were deposited under the numbers **ph005_48**, **ph005_49**, **ph006_04** respectively in the herbarium of the Department of Biology, University of Badji Mokhtar-Annaba, Algeria.

Plants were air dried at room temperature, ground on mill to obtain powder and stored in a glass flask to protect them from humidity and light.

Chemicals

Folin-Ciocalteu's phenol reagent, aluminum chloride, quercetin and vitamin C were purchased from Acros Organics. Potassium persulfate, sodium carbonate, sodium nitrite, hydrochloric acid, sodium hydroxide, methanol and DPPH were obtained from Sigma and Roth (France). The chemicals used were all of analytical grade.

Phytochemical screening

Preliminary tests for determination of secondary metabolites were carried out according to the Harborne technique [13].

Extraction procedure

Material plants were macerated with methanol, ethanol, MeOH-H_2O (70/30 v/v), and H_2O for 24 h three times. The organic phases were filtered through Whatman filter paper No1, concentrated under reduced pressure to dryness. Then stocked under 4°C for analyses.

Dosages of phenolic compounds and antioxidant activity

otal phenol contents

Total phenolic contents were evaluated with Folin-Ciocalteu's phenol reagent [14-15] using the spectrophotometric analyses (Cary 50 Scan UV-Visible apparatus). Briefly, an aliquot (1 mL) of standard solutions of Gallic acid at different concentrations or appropriately diluted extracts was added to a 25 mL volumetric flask containing 9 mL of ddH_2O. A reagent blank using ddH_2O was prepared, 1 mL of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 mL of 7% Na_2CO_3 solution was added with mixing. The solution was then immediately diluted to volume (25 mL) with ddH_2O and mixed thoroughly. After incubation for 90 min at 23°C, the absorbance versus prepared blank was read at 750 nm. Total phenolic content was expressed as mg Gallic acid equivalents (GAE)/100g dry matter. Samples were analyzed in three replications.

Total flavonoid contents

Total flavonoid content was measured according to $AlCl_3$ method [16]. A 1 mL aliquot of standard solutions of quercetin at different concentrations or appropriately diluted samples was added to a 10 mL volumetric flask containing 4 mL ddH_2O. At zero time, 0.3 mL 5% $NaNO_2$ was added to the flask. After 5 min, 0.3 mL 10% $AlCl_3$ was added. At 6 min, 2 mL of 1 M NaOH was added to the mixture. Immediately, the solution was diluted to volume (10 mL) with ddH_2O and mixed thoroughly. Absorbance of the mixture, pink in color, was determined at 510 nm versus the prepared blank. Total flavonoid content was expressed as mg quercetin equivalents (QE)/100g dry weight (dw). Samples were analyzed in three replications.

Antioxidant activities

The DPPH radical scavenging assay

The antioxidant activity of plant extract was evaluated using a slight modification of the DPPH radical scavenging protocol described by Chun et al. [17].

2.9 mL of a 0.004% MeOH solution of DPPH was mixed with 0.1 mL of plant extract. The mixture was shaken and incubated at 20°C in the dark for 40 min and thereafter the decrease of the optical density was recorded at 517 nm against the blank. For the control, 2.9 mL of a 0.004% MeOH solution of DPPH was mixed with 0.1 mL of methanol, and optical density of solution at 517 nm was recorded after 30 minutes. Vitamin C equivalent antioxidant capacity (VCEAC) was calculated by using ascorbic acid as a reference compound to prepare the standard curve and the DPPH radical scavenging activity of phenolic compounds were expressed in terms of mg of vitamin C per 100 g of dry matter (VCEAC).

The radical-scavenging activities of the extracts, expressed as percentage inhibition of DPPH (%IP), were calculated according to equation 1:

$$\%IP = [(A_0 - A_1)/A_0] \times 100 \qquad (1)$$

Where A_0 is the absorbance of the control reaction mixture, and A_1 is the absorbance of the extract/standard after 20 minutes. The amount of sample needed to decrease the initial DPPH concentration by 50% (IC_{50}) was calculated graphically by using the curve drawn for inhibition percentage versus used concentration and was expressed in terms of mg/L of dry weight. The radical solution was prepared daily and samples were analysed in three replications.

The ABTS scavenging assay

For ABTS assay, the procedure followed was the method of Roberta [18] with some modifications. ABTS was dissolved in water to make a concentration of 7 mmol/L. $ABTS^{+}$ was produced by reacting the ABTS stock solution with 2.45 mmol/L potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room

temperature for 12–16 h before use. For the test of samples, the $ABTS^{+}$ stock solution was diluted with 80% methanol to an absorbance of 0.70 ± 0.02 at 734 nm.

Vitamin C was used as a reference compound. The results were expressed as vitamin C equivalent antioxidant capacity (VCEAC) values and calculated as mean value ± standard deviation (SD) (n = 3).

Antimicrobial Activity

Microorganisms tested

The following strains of bacteria were used as test microorganisms: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae*, *Salmonella enteritidis*, *Candida albicans*, and *Staphylococcus aureus* (ATCC 25923).

These microorganisms were obtained from Microbiology Laboratory, Faculty of Medicine, Badji Mokhtar University.

Preparation of tested samples

The different dried extracts were respectively dissolved in sterile (DMSO) and sterile water at selected concentrations (50 mg/mL, and 150 mg/mL). The antibacterial activity of the extracts was carried out by the disc diffusion method [19-21].

The agar gel (Mueller-Hinton agar) is treated with the appropriate microorganism suspension (each microorganism was inoculated a concentration of ca 10^7 colony forming units per ml. The assays are based on the use of sterile discs filter paper (6 mm diameter) impregnated with 20 µl of the plant extract solution to be examined and allowed to dry at room temperature. A sterile disc impregnated with DMSO is used as negative control. After incubation for 24 h at 37°C, all plates were observed for zone of growth inhibition and the diameters of these zones were measured in millimeters. All experiments were carried out in triplicates.

Statistical analysis

All analyses were carried out in triplicates. The results of scavenger activity, total phenolic and total flavonoid contents were performed from the averages of all samples reading mean ± standard deviation using Excel 2007 and STATISTICA software on student one Test. Observed differences were statistically considered significant at the level of $P < 0.05$.

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screening revealed the presence of many chemical groups and the results are shown in table 1.

Table 1. Phytochemical screening of plants

Family compound	*E. terracina* (a.p)	*E. biumbellata* (a.p)	*E. dendroides* (a.p)
Alkaloids	-	-	-
Flavonoids	++	++	++
Tannins	++	++	++
Cardenolides	+	+	-
Sterols and terpenes	+	+	+
Saponins	+	+	+
Lipids	+	+	+
Anthocyanins	-	-	+

a.p: aerial parts; + : Positive; ++ : Strongly positive; - : Not detected

The phytochemical analysis revealed the presence of several chemical families in the aerial parts of the plants studied (table 1): tannins, flavonoids, lipids, sterols, saponins and terpenes. In contrast, the alkaloids were not detected. These results were previously reported by Özbilgin et al., Noori et al. and Gihan et al. [12,22,23].

It is concluded from the data of the phytochemical screening that only *E. dendroides* contains anthocyanins. On the other hand, cardenolides only found in the *E. biumbellata* and *E. terracina*.

Determination of total phenol contents and total flavonoid contents

The results obtained of total phenolic and flavonoids contents of studied plants are expressed in table 2 and 3 and illustrated by figure 1 and 2.

Table 2. Total phenol contents (GAE g/100g dw).

Plant part	MeOH	H_2O	MeOH-H_2O (7/3)	EtOH
E.B.L	15.13	14.33	15.79	12.695
E.B.S	3.715	3.13	3.88	2.435
E.D.L	6.93	5.39	7.55	5.993
E.D.S	3.035	2.955	3.215	1.84
E.T.L	5.472	4	5.52	3.77
E.T.S	2.301	2.5	3.18	2.09

E.B.L: *Euphorbia biumbelLata* Leaves ;**E.B.S**: *Euphorbia biumbelLata* seeds ;**E.D.L**: *Euphorbia dendroides* Leaves ;**E.D.S**: *Euphorbia dendroides* seeds ;**E.T.L**: *Euphorbia terracina* Leaves ;**E.T.S**: *Euphorbia terracina* seeds .

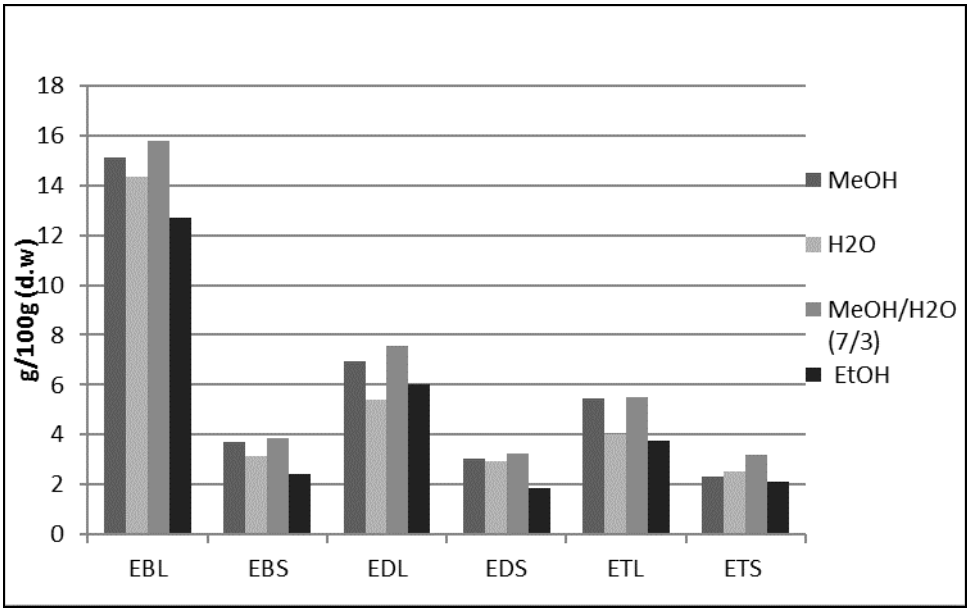

Figure 1: Total phenolic content of three plants

E.B.L: *Euphorbia biumbelLata* Leaves ;**E.B.S**: *Euphorbia biumbelLata* seeds ;**E.D.L**: *Euphorbia dendroides* Leaves ;**E.D.S**: *Euphorbia dendroides* seeds ;**E.T.L**: *Euphorbia terracina* Leaves ;**E.T.S**: *Euphorbia terracina* seeds .

Table 3. Total flavonoid contents (QE g/100g dw).

Plant part	MeOH	H_2O	MeOH-H_2O (7/3)	ETOH
E.B.L	5.292	5.006	7.06	3.993
E.B.S	2.973	2.794	3.093	2.086
E.D.L	5.132	4.72	6.754	3.62
E.D.S	2.846	2.266	2.98	1.926
E.T.L	4.856	3.716	4.955	3.096
E.T.S	2.301	1.917	2.6845	1.52

E.B.L: *Euphorbia biumbelLata* Leaves ;**E.B.S**: *Euphorbia biumbelataa* seeds ;**E.D.L**: *Euphorbia dendroides* Leaves ;**E.D.S**: *Euphorbia dendroides* seeds ;**E.T.L**: *Euphorbia terracina* Leaves ;**E.T.S**: *Euphorbia terracina* seeds .

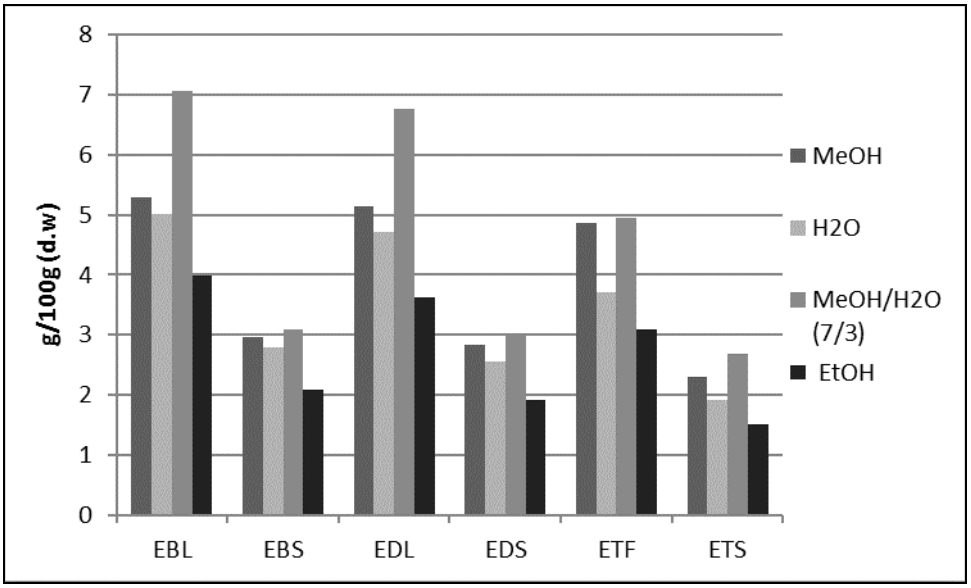

Figure 2: Total Flavonoid Content of different plants

E.B.L: *Euphorbia biumbellata* Leaves ;**E.B.S**: *Euphorbia biumbellata* seeds ;**E.D.L**: *Euphorbia dendroides* Leaves ;**E.D.S**: *Euphorbia dendroides* seeds ;**E.T.L**: *Euphorbia terracina* Leaves ;**E.T.S**: *Euphorbia terracina* seeds .

The results revealed that (7/3: MeOH / H_2O) extract of *E. biumbellata* leaves was found to have the highest content of both total phenolic compound and total flavonoids with 15.79 g of gallic acid equivalents, and 7.06 g of quercetin equivalents per 100g of dry matter respectively. The lowest amount of total phenolic and flavonoids content is recorded in ethanol extract of *E. terracina* seeds with 2.09 g GAE, and 1.52 g QE per 100g of dry matter.

Antioxidant capacity

Radical scavenging activity by DPPH

The results of the antioxidant capacity of studied plants, determined by DPPH test are shown in table 4.

Table 4. The antioxidant capacity by DPPH

A.P	ET	ED	EB
C(mg/L)	19.736	26.1	26.638
VCEAC(g/100g)	0.7285	1.044	1.0655

A.P: aerial parts ;**E.B**: *Euphorbia biumbellata* ;**E.D**: *Euphorbia dendroides* ;**E.T**: *Euphorbia terracina* ,

VCEAC(g/100g): g of Vitamin C equivalent antioxidant / 100g of dry matter.

The radical scavenging activity of the methanol extract, determined by DPPH test; expressed as mg of vitamin C equivalent (VCEAC) per 100 g of plant extract are shown in figure 3.

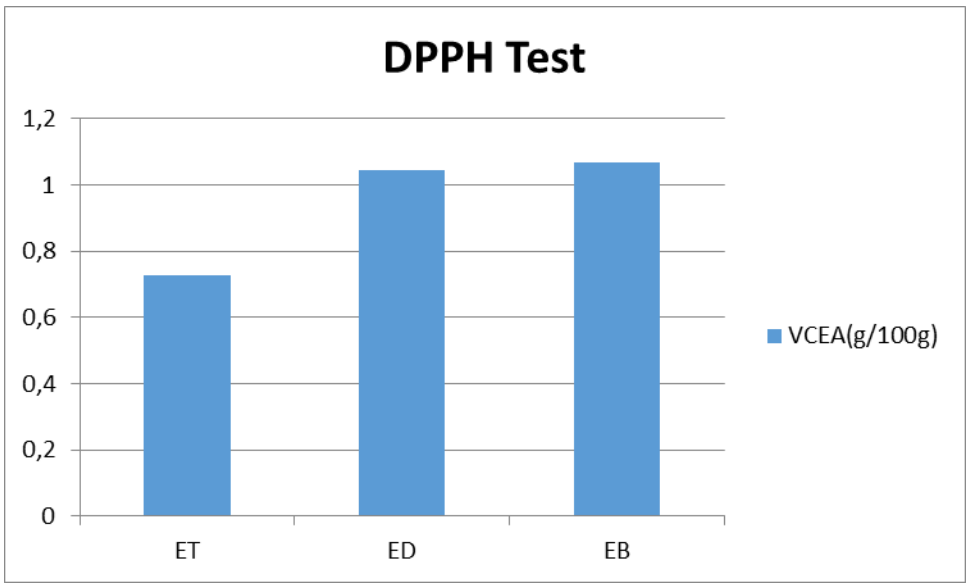

Figure 3: Vitamin C equivalent antioxidant capacity (VCEAC) by DPPH

E.B: *Euphorbia biumbellata* ;**E.D**: *Euphorbia dendroides* ;**E.T**: *Euphorbia terracina* .

The test revealed that the methanol extract of *E. biumbelleta* possessed the highest antioxidant capacity of 1.065 g VCEAC/100 g of plant extract, followed by methanol extract of *E. dendroides* of 1.044g VCEAC/100 g of plant extract. These results are confirmed by IC50 values calculated through the interpolation of linear regression analysis. *E. biumbellata* showed lowest DPPH based IC_{50} (49 mg/mL) while *E. terracina* (84 mg/mL) had the highest IC_{50} value.

A lower value corresponds to a higher antioxidant activity of the plant extract.

Otherwise, a significant correlation between total flavonoid content and antioxidant activity is observed ($R^2 = 0.96$).

Radical scavenging activity by ABTS

The results of the antioxidant capacity of studied plants, determined by ABTS test are shown in table 5.

Table 5. The antioxidant capacity of aerial part of plants by ABTS

A.P	EB	ED	ET
C(mg/L)	0,374	0,336	0,031
VCEAC(g/100g)	1,403	0,725	0,640

A.P: Aerial parts ;**E.B**: *Euphorbia biumbellata* ;**E.D**: *Euphorbia dendroides* ;**E.T**: *Euphorbia terracina* ,

VCEAC(g/100g): g of Vitamin C equivalent antioxidant / 100g of dry matter.

The radical scavenging activity of the methanol extract, determined by ABTS test; expressed as mg of vitamin C equivalent (VCEAC) per 100 g of plant extract are shown in figure 4.

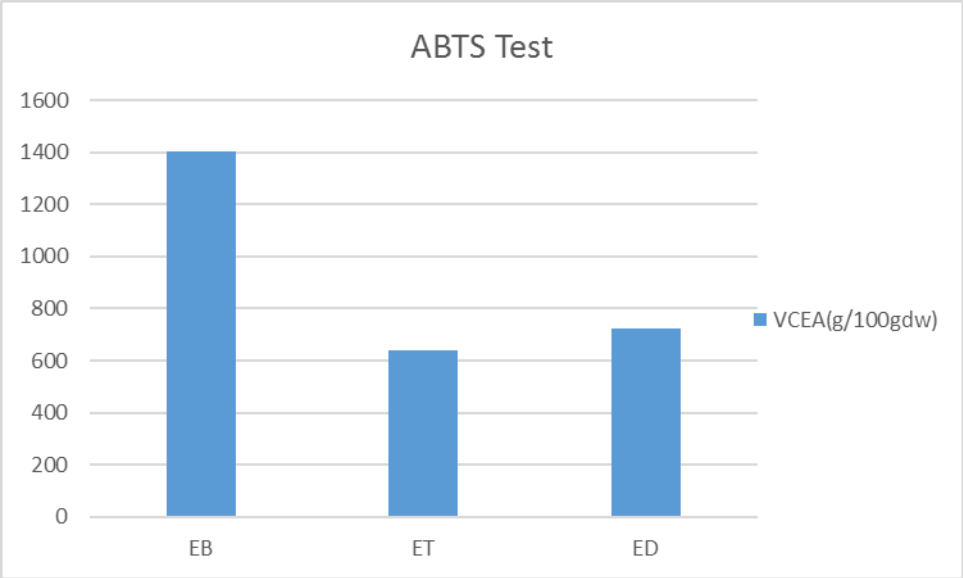

Figure 4: Vitamin C equivalent antioxidant capacity (VCEAC) by ABTS

E.B: *Euphorbia biumbellata* ;**E.D**: *Euphorbia dendroides* ;**E.T**: *Euphorbia terracina* .

The methanol extract of *E.biumbellata* displayed the highest radical scavenging potential (1.403 g VCEAC/100 g). The methanol extract of *E. terracina* had the lowest antioxidant capacity of (0.64 g VCEAC/100 g).

Antimicrobial activity

The results for the general screening for antibacterial activity are shown in table 6.

Table 6. *In vitro* antibacterial activity of methanolic extracts from three plants

	Inhibition zone (mm)					
B.S	*E.c*	*S.a*	*P.a*	*S.e*	*K.p*	*C .a*
C(mg/mL)	150	150	150	150	150	150
E.B.L	22	22	16	22	15	16
E.B.S	21	10	10	19	11	13
E.D.L	20	12	14	18	18	15
E.D.S	15	7	8	19	12	11
E.T.L	22	10	12	15	16	18
E.T.S	19	8	18	18	15	14

S.B : Bacterial strains **; S.d :** *Salmonella enteritidis***; S.a** : *Staphylococcus aureus* **; E.c** : *Escherichia coli***; P.a** : *Pseudomonas aeruginosa*; **C.a** : *Candida albican*; **K.p** : *Klebsiella pneumoniae*; **E.B.L**: *Euphorbia biumbellata* leaves; **E.B.S**: *Euphorbia biumbellaa* seeds; **E.D.L**: *Euphorbia dendroides leaves*; **E.D.S**: *Euphorbia dendroides* seeds; **E.T.L**: *Euphorbia terracina* leaves; **E.T.S**: *Euphorbia terracina* seeds.

Six extract fractions of different parts belonging to three plant species were investigated.

The three plants were active and showed a relatively high level of antibacterial activity, The highest activity (diameter of zone of inhibition 22mm and MIC 0.2mg/mL) was demonstrated by the the methanol extract of *E. biumbellata* leaves against *Escherichia coli* and *Staphylococcus aureus* while the lowest activity (diameter of zone of inhibition 7mm and MIC 6.25mg/mL) was demonstrated by the methanol extract of *E. dendroides* seeds against *Staphylococcus aureus.*

The results of present investigation clearly indicate that the antibacterial activity vary with the species of the plants, and also showed that the leaf extracts are more effective than the seed extracts.

CONCLUSION

In conclusion, the aim of the present study was to evaluate the antimicrobial and antioxidant capacity of three toxic plant extracts by using DPPH scavenging test and ABTS method and to determine the phenolic and flavonoid contents. The results indicate that all the plants exhibited potent antioxidant activity. The presence of flavonoids and tannins in all the plants is likely to be responsible for the free radical scavenging effects observed [24]. The methanol extract of *Euphorbia biumbellata* possesses a high antioxidant activity. In other hand, the different extracts were relatively active against the tested microorganisms; activity has been displayed by *Euphorbia biumbellata* and *Euphorbia dendroides* leaf extract against *Salmonella enteritidis* and *E.coli*. The antioxidant activities and antimicrobial activities are correlated for studied plants.

More biological studies to explore the activity against more pathogenic organisms and toxicological investigation should be considered. Additionally, the interest in isolation of pure agents is planning.

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